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Selection strategies for increasing the yield of high nutritional value leaf mass in *Urochloa* hybrids

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Abstract Experimental genotype selection in a forage crop cultivar development program is complex because the ultimate aim is increase performance and sustainability in animal production. The objectives of this study were (1) to identify traits with greater direct and indirect effects on yield of high nutritional value leaf mass (NLM) in *Urochloa* sp. hybrids and (2) to assess indirect gain from selection for these traits with greater effects through selection indexes and by genotype by yield*trait (GYT) biplot analysis using NLM as a basic variable. We evaluated 96 interspecific hybrids, from a gene pool among *Urochloa ruziziensis*, *Urochloa brizantha* and *Urochloa decumbens* species, in an experiment laid out in a randomized complete block design. A series of agronomic and nutritional value traits were measured. Path analysis and GYT were performed using NLM as the basic variable, and different strategies using selection indexes were adopted. The leaf dry matter and field green weight (FGW) traits exhibited greater direct effects on NLM. All selection strategies proved to be effective in obtaining gains in the NLM variable. GYT analysis and the selection index with weights corresponding to the relative direct effects to each trait on the NLM were the strategies that resulted in a greater correlated response for NLM. Indirect selection for NLM via FGW or the index with the FGW and regrowth capacity traits proved to be viable strategies for selection of *Urochloa* genotypes in the initial stages of the breeding cycles due to their practicality and lower requirement regarding traits to be measured.

Keywords *Urochloa* · Genetic correlation · Indirect selection · Path analysis · Index selection · Biplot analysis

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Introduction

Cultivated pastures are the main and the most economic source to feed animals in the Brazil, which the *Urochloa* genus stands out because it is the most used tropical forage grass genus in cultivated pasture area in the country (Jank et al. 2014). In the interspecific breeding program of *Urochloa,* the crosses began in the late 1980s using apomictic

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genotypes of *U. decumbens* or *U. brizantha*, as pollen donors, and artificially tetraploidized sexual genotypes of *U. ruziziensis*. These three species belong to the same agamic complex and form a gene pool (Miles 2007; Worthington and Miles 2015). The crosses between apomictic and sexual parents result progenies segregating 1:1 for reproductive mode, being the apomictic genotypes candidate to new cultivars whereas superior sexual genotypes are selected as new parents in a recurrent selection method (Jank et al. 2014; Worthington and Miles 2015). The breeding program of *Urochloa*, just as for other genera of forage species, aim to improve several traits simultaneously, such as agronomic and nutritional value traits. However, the selection of cultivars in forage species is more complex than annual row crops because the final aim is the gain in animal performance, such as meat or milk production (Valle et al. 2008).

In the initial phases of a forage breeding program, the use of animals for selection of the best genotypes is not feasible, due to the large number of genotypes under testing (e.g., progenies, hybrids). In this case, agronomic traits have been measured and chemical analyses have been performed by near-infrared spectroscopy (NIRS) for the purpose of estimating the nutritional value of the best genotypes (Mateus et al. 2015; Matias et al. 2016). These evaluations do not provide values of leaf consumption, digestibility, and protein content available for the animal because there are other factors that affect consumption. These factors include plant architecture, pasture management, and/or anti-nutritional factors, for example, "girder" structures that increase resistance to digestion by the animal (Wilson et al. 1989), as well as factors intrinsic to the animal, such as age and breed. However, these initial evaluations are useful for discrimination of the genotypes and for better understanding of the associations between these variables and animal consumption, which is fundamental for successful development of cultivars.

Cultivars of forage species that have high leaf yield and good nutritional quality, such as high digestibility and protein content, are related to better animal performance (Euclides and Euclides Filho 1998; Torres et al. 2015, 2016). Since animal performance is an indirect product of leaf dry matter production, protein content, and digestibility, an index composed simultaneously of all these traits was proposed in this study, i.e., the yield of high nutritional value leaf mass, with the aim of selecting superior genotypes. It is important highlight that this index does not quantify the digestible protein yield because the digestibility measured refer to leaf organic matter digestibility.

Currently in the forage breeding program, measurement of most traits evaluated in *Urochloa* is through morphological separation (leaf blade, stem + leaf sheath, and dead material) of forage samples, drying, grinding of samples, and then analysis by NIRS, which consumes time and resources (Mateus et al. 2015; Matias et al. 2016). Thus, correlations among traits are of great importance because they assist in choosing the selection strategies to be used in the breeding program. Although the correlations quantify the magnitude and direction of the association between two traits, they do not provide the exact relative importance of the direct and indirect effects of these traits (Cruz et al. 2012). In addition, simple correlation estimates might not represent the true association between two traits since there may be interference from a third trait, or group of traits, that may skew the correlation estimates (Wright 1921). Several factors influence this parameter as such as species, population, environment and estimation method, and so, in the literature there are contrasting correlation estimates for some traits in *Urochloa*, as for example, 0.97 and 0.32 between total dry matter (TDM) and regrowth capacity (REG), 0.00 and − 0.71 between TDM and in vitro digestibility of organic matter (IVD), and − 0.22 and 0.50 between TDM and neutral detergent fiber (NDF) (Figueiredo et al. 2012; Matias et al. 2016).

Path analysis, proposed by Wright (1921), arose with the aim of eliminating the limitations of simple correlation analysis. Path analysis allows decomposition of the simple correlation coefficients in direct and indirect effects of a group of traits on a basic variable of interest (Cruz et al. 2014). This type of analysis has been used in breeding of various crops, such as maize and soybean (Alves and Cargnelutti Filho 2017; Kmail et al. 2017; Machado et al. 2017). However, there are few studies on path analysis in *Urochloa* (Borges et al. 2011; Torres et al. 2016).

Another difficulty in forage breeding, just as in other crops, is selection for multiple traits, because this selection has generally been dependent on a subjective weight given to each trait when using a selection index (Mateus et al. 2015; Yan and Frégeau-Reid 2018). Aiming to overcome this problem, Yan and Frégeau-

Reid (2018) proposed a new approach of analysis for multiple traits, the genotype by yield*trait (GYT) biplot analysis. In this analysis, there is no attribution of weights to the traits, and the ranking of the genotypes is based on the combination of the basic variable (e.g., yield) with other primary and secondary breeding targets.

Therefore, the aims of this study were (1) to identify traits with greater direct and indirect effects on yield of high nutritional value leaf mass (NLM) in *Urochloa* hybrids and (2) to assess indirect gain from selection for these traits with greater effects through selection indexes and by genotype by yield*trait (GYT) biplot analysis using NLM as a basic variable.

Materials and methods

Genotypes and phenotypic data

We evaluated a total of 99 genotypes: 96 interspecific hybrids of *Urochloa* (gene pool from *U. ruziziensis*, *U. brizantha* and *U. decumbens* species), selected from a population composed of 1,000 hybrids obtained by a partial diallel (Matias et al. 2018), and the checks *U. brizantha* cv. Marandu, *U. brizantha* cv. BRS Paiaguás, and the interspecific hybrid cv. Mulato II. The experiment was conducted in the experimental field of Embrapa Beef Cattle, CNPGC, in Campo Grande, Mato Grosso do Sul, Brazil (20° 27′ S, 54° 37′ W, and altitude of 530 m AMSL). Seedlings selected, based agronomic performance and spittlebug resistance, from individual plants of the population trial (Matias et al. 2018) were transplanted into tubes containing substrate (mixture of sand, organic compound and subsoil soil in a 1:1:1 ratio). These seedlings were kept in a greenhouse for 60 days until these to be transplanted to the field. The fertilization in the field was made based on soil analysis in order to reach the recommendations for *U. brizantha* cv. Marandu, which is: pH from 5.5 to 6.5, base saturation from 40 to 50%, Ca saturation from 30 to 45%, Mg saturation from 10 to 15%, $P > 5$ mg dm^{-3}, $K > 0.13$ cmol$_c$ dm^{-3}, $S > 10$ mg dm^{-3}, $B > 0.5$ mg dm^{-3} and $Zn > 1.5$ mg dm^{-3}. In addition, 45 kg ha^{-1} of N was applied. The trial was set up on 18 Nov. 2014 in a randomized complete block design with four replications; plots consisted of five clonal plants at a spacing of 0.5 m within rows and 1.0 m between rows.

Seven cuttings were made (cutting 1: 03 Feb. 2015, cutting 2: 10 Mar. 2015, cutting 3: 23 Apr. 2015, cutting 4: 13 July 2015, cutting 5: 13 Oct. 2015, cutting 6: 25 Nov. 2015, and cutting 7: 18 Jan. 2016), in which agronomic and nutritional value traits were evaluated. The agronomic traits evaluated were field green weight (FGW, kg ha^{-1}), regrowth capacity (REG, score), total dry matter yield (TDM, kg ha^{-1}), leaf dry matter yield (LDM, kg ha^{-1}), leaf percentage (%L), and leaf/stem ratio (LSR). In each plot, each cutting was weighed in the field with a dynamometer, to measure FGW. REG was evaluated 7 days after the cutting through a scoring scale, as described by Figueiredo et al. (2012). A sample was removed from each plot in the cuttings, and these samples were dried in laboratory ovens at 65 °C for 72 h to estimate TDM. In cuttings 2, 5, and 7, the samples were separated morphologically (leaf blade, stem + leaf sheath, and dead material), thus allowing estimation of %L, LDM, and LSR. The FGW and REG traits were evaluated in all the cuttings, and TDM in six cuttings (except in cutting 1).

To assess nutritional value traits, the dry leaves from morphological separation of cuttings 2, 5, and 7 were ground and analyzed using calibration curves via NIRS (Marten et al. 1989). The calibration of the NIRS was performed previously by comparing the results obtained in the chemical analyses and the spectrum read from these same samples in the NIRS for several nutritional traits, as described by Matias et al. (2018). The following nutritional traits were measured: crude protein in dry matter (CP, %, AOAC 1990), in vitro digestibility of organic matter (IVD, %, Tilley and Terry 1963), percentage of neutral detergent fiber (NDF, %, Van Soest et al. 1991), and percentage of lignin in sulfuric acid in the dry matter (LIG, %, Van Soest et al. 1991). The yield of high nutritional value leaf mass (NLM, kg ha^{-1}) was also estimated. This variable was estimated by the following equation: NLM = LDM × CP × IVD. The NLM was estimated for three cuttings (2, 5, and 7), the same cuttings in which LDM, CP, and IVD were measured.

Statistical analysis

The multi-cutting analysis of the cuttings was conducted on the data through the mixed model approach,

considering kinship information of the sexual progenitors, with estimation of variance components using the residual maximum likelihood (REML) method through the ASReml statistical package of R (Butler 2009). Combined analysis of each trait used the following model:

$$y = Xm + Z_1 b + Z_2 g + e$$

where y is the phenotypic data vector; X is the incidence matrix of the fixed effects and the Z's are the incidence matrices in reference to the random effects; m is the fixed effects vector of cuttings, which are added to the overall mean; b is the random effects vector of blocks, in which $b \sim NMV\left(0, I\sigma_b^2\right)$ and σ_b^2 is the variance component associated with the effects of blocks; g is the random effects vector of genotypes within cuttings, in which $g \sim NMV(0, G \otimes A)$; and e is the random error vector within cuttings, in which $e \sim NMV(0, R \otimes I)$; R is the residual covariance matrix; G is the genetic covariance matrix; A is the relationship (kinship) matrix; and I is the identity matrix. The Kronecker product is denoted by $\otimes$. For each trait, the R and G matrices were chosen in a sequential manner (Smith et al. 2007; Andrade et al. 2016), first identifying the best covariance structure for R and then for G, considering the previously selected R. The best structure for R and G was indicated by the lowest value of the Bayesian Information Criterion (BIC), proposed by Schwarz (1978).

The significance of the variance components was checked by the likelihood ratio test (LRT) at 5% probability. The BLUP predictions of the random effects were obtained, and then the genetic correlations between the traits were estimated using the BLUPs of the hybrids using the *Agricolae* R package (Mendiburu 2014). The significance of the genetic correlations was checked via Bootstrap by the "bias-corrected and accelerated" (BCa) method, with 9999 simulations, using the *wBoot* R package (Weiss 2016). Genotype mean-based heritability and selective accuracy were also estimated, according to Resende and Duarte (2007).

Path analysis

The multicollinearity test was performed through evaluation in the number of conditions (NC) proposed by Montgomery and Peck (1981), which examines the relation between the highest and lowest eigenvalue of the genetic correlation matrix. Multicollinearity is considered low with NC $<$ 100, moderate to strong if 100 $>$ NC $>$ 1000, and severe if NC $>$ 1000. Multicollinearity was diagnosed using the Genes software (Cruz 2013), and when multicollinearity was above to moderate, a constant of 0.035 was added in the diagonal of the correlation matrix, in a way similar to the ridge regression method (Carvalho and Cruz 1996). Path analysis was carried out using a two-chain causal model, with the NLM trait as the basic variable; CP, IVD, NDF, LIG, and LDM, the primary variables; and FGW, TDM, %L, LSR, and REG, the secondary variables (Fig. 2). The causal diagram was chosen based on a theoretical knowledge of causal relationships between traits.

Genotype by yield*trait biplot

For the genotype by yield*trait biplot analysis, the NLM variable was taken as the basic or main variable (yield). From this, the BLUP means of the hybrids in regard to NLM from combined analysis of the cuttings were combined with the BLUP means of each one of the other traits for each hybrid to obtain the values of the yield*trait variables, as proposed by Yan and Frégeau-Reid (2018). For FGW, TDM, LDM, %L, LSR, REG, CP, and IVD, in which selection is to increase the trait, the BLUP of the trait was multiplied by the BLUP of NLM for each hybrid, for example, NLM*CP. For LIG and NDF, in which selection is to reduce the value of the trait, the BLUP of NLM was divided by the BLUP of these traits, i.e., NLM/NDF and NLM/LIG. Thus, higher values of GYT are always desirable. Biplots of the yield*trait variables were generated using the *GGEBiplotGUI-R* package (Frutos et al. 2014).

Selection strategies

For selection of the genotypes, six selection strategies were used aiming to increase the NLM trait: strategy 1—direct selection for NLM; strategy 2—indirect selection based on the FGW trait; strategy 3—indirect selection by means of the selection index, with the FGW and REG traits with predefined weights of 60% and 40%, respectively; strategy 4—indirect selection by means of the selection index including all the traits, except NLM, with predefined weights of 60% for the

agronomic traits and 40% for those of nutritional value (the traits had equal weights within each category); strategy 5—indirect selection by means of the selection index including all the traits, except NLM, with weights corresponding to the relative direct effects related to each trait on the NLM trait from path analysis; and strategy 6—selection based on GYT analysis, which does not require the establishment of weights, according to Yan and Frégeau-Reid (2018). To compare the different selection strategies, gain from selection (%) was calculated using the selection intensity of 10%, and the *Spearman* correlation of the genotypes among the selection strategies was calculated using the *Agricolae*-R package (Mendiburu 2014).

Results

Genetic parameters

The covariance structures of the *G* and *R* matrices varied according to the traits evaluated. The best structure of the *R* matrix was the unstructured (UN) for FGW, TDM, and CP; compound symmetry (CS) for REG; heterogeneous compound symmetry (CSH) for %L and LIG; and first order autoregressive with heteroscedasticity (ARH1) for LDM, LSR, IVD, NDF, and NLM. As for the *G* matrix, the structure that provided the lowest BIC was ARH1 for FGW, CSH for LSR and LDM, and CS for the other traits.

The genetic variance was significant by the LRT for all the traits evaluated. The variance of the genotype x cutting interaction ($(\sigma^2_{G\times C})$ was non-null for several of the traits as well, except for CP, IVD, NDF, and LIG (Table 1). Genotype mean-based heritability had a magnitude ranging from 31 (%L) to 78% (REG), and for most of the traits, the estimated values were above 51% (Table 1). The heritability estimates were lower than 50% only for %L (31%), IVD (48%), and LIG (45%), which denotes the greater influence of environmental factors on phenotypic expression of these traits. The estimates of selective accuracy were of high magnitude for most of the traits (Table 1), except for %L (55%), IVD (70%), and LIG (67%), which had estimates of moderate magnitude, according to Resende and Duarte (2007). In general, this displays good reliability in the selection of genotypes under testing.

Significant genetic correlations were detected among most of the traits evaluated (Fig. 1). The correlations ranged from 0.93 (NLM–LDM) to − 0.42 (IVD–NDF). The most highly correlated traits were NLM × LDM (0.93), FGW × LDM (0.85), TDM × LDM (0.86), FGW × TDM (0.84), FGW × NLM (0.83), LSR × %L (0.79), and TDM × NLM (0.77). The correlations among the nutritional value traits, and the nutritional value traits with the agronomic traits or NLM were of moderate to low magnitude.

Path analysis

The genotypic correlation matrix exhibited moderate to strong multicollinearity, detected by the test proposed by Montgomery and Peck (1981). In this case, path analysis was carried out considering multicollinearity, as suggested by Carvalho and Cruz (1996). In analysis of the primary variables on the main variable, an R^2 of 0.94 and an effect of the residual variable (ERV) of 0.25 were observed, indicating that most of the variation was explained by these variables. The LDM had a high correlation estimate and greater direct effect on the main variable NLM. The other traits had a direct effect lower than the ERV, as well as low correlation with NLM (Fig. 2).

Considering the effects of the secondary variables on the primary variables, an R^2 and ERV of 0.05 and 0.97 were observed for CP, 0.14 and 0.92 for IVD, 0.22 and 0.88 for NDF, and 0.38 and 0.78 for LIG, respectively. Thus, these secondary variables did not satisfactorily explain all the variation for CP, IVD, NDF, and LIG. However, for LDM, they were able to explain a considerable part of the variation (R^2 = 0.90), and the ERV was 0.32. The TDM and FGW traits had not only a high positive correlation, but also a direct positive effect on LDM (Fig. 2). Analysis of the effects of the secondary traits on NLM (R^2 = 0.77 and ERV = 0.47) showed that FGW had a high magnitude correlation and high direct effects on NLM (Fig. 2). TDM had a correlation estimate of 0.77, but with a direct effect lower than ERV. In a similar manner, REG had a moderate correlation estimate with NLM and a small direct effect (Fig. 2).

Table 1 Estimates of variance of genotypes (σ_g^2), variance of the genotype × cutting interaction ($\sigma_{g\times c}^2$), genotype mean-based heritability (h^2, %), accuracy ($r_{\hat{g}g}$, %), and overall mean ($\bar{Y}_{overall}$) for agronomic traits and nutritional value traits in *Urochloa* hybrids in multi-cutting analysis[a]

	FGW		TDM		LDM		%L	
	Mean variance	LRT	Variance	LRT	Variance	LRT	Variance	LRT
$\hat{\sigma}_g^2$	10695503.00	260.8*	185950.55	42.9*	117252.80	8.9*	6.31	4.2*
$\hat{\sigma}_{g\times c}^2$	–	–	32520.35	23.3*	55501.82	9.3*	20.81	51.2*
h^2	73		65		63		31	
$r_{\hat{g}g}$	85		81		80		55	
$\bar{Y}_{overall}$	12343.60		2676.91		1762.96		63.62	
	LSR		REG		CP		IVD	
	Mean variance	LRT	Variance	LRT	Variance	LRT	Variance	LRT
$\hat{\sigma}_g^2$	0.36	85.4*	0.22	145.9*	0.48	75.8*	2.36	12.2*
$\hat{\sigma}_{g\times c}^2$	–	–	0.13	122.4*	0.01	0.4^{NS}	0.00	0.0^{NS}
h^2	72		78		75		48	
$r_{\hat{g}g}$	85		88		87		70	
$\bar{Y}_{overall}$	3.85		2.86		13.50		63.81	

	NDF		LIG		NLM	
	Variance	LRT	Variance	LRT	Mean variance	LRT
$\hat{\sigma}_g^2$	0.99	16.4*	0.02	11.8*	1609.55	105.1*
$\hat{\sigma}_{g\times c}^2$	0.22	0.8^{NS}	0.00	0.0^{NS}	–	–
h^2	54		45		69	
$r_{\hat{g}g}$	74		67		83	
$\bar{Y}_{overall}$	68.01		2.11		146.31	

FGW = field green weight (Kg ha^{-1}), *TDM* = total dry matter yield (Kg ha^{-1}), *LDM* = leaf dry matter yield (Kg ha^{-1}), *%L* = leaf percentage (%), *LSR* = leaf/stem ratio, *REG* = final regrowth capacity (score), *CP* = crude protein contents in dry matter (%), *IVD* = in vitro digestibility of organic matter (%), *NDF* = percentage of neutral detergent fiber (%), *LIG* = percentage of lignin in dry matter (%), *NLM* = yield of high nutritional value leaf mass (Kg ha^{-1})

* and ^{NS} Significant and non significant by the likelihood ratio test (LRT) at 5% probability, respectively. [a]Seven cuttings: FGW, REG; six cuttings: TDM; three cuttings: LDM, %L, LSR, CP, IVD, NDF, LIG, and NLM

Selection strategies

Direct selection for NLM (strategy 1), as expected, led to the greatest gain for this trait (34%) (Table 2). Selection based only on FGW (strategy 2) or FGW plus REG (strategy 3) led to high and favorable gains for NLM, of 29% and 27%, respectively (Table 2). However, unfavorable gains were found for LSR and LIG with strategy 2 and for CP and LIG with strategy 3, and null gains for IVD with these both strategies (Table 2). The Spearman correlation estimates of these strategies with strategy 1 were 0.82 and 0.83, respectively (Table 3). The strategies based on selection indexes in which all the traits, except NLM, were included (strategies 4 and 5), led to gains greater than those achieved from strategies 2 and 3, but also exhibited unfavorable gains for LIG (Table 2). The Spearman correlation estimate between strategy 6 and direct selection for NLM was 0.99.

The checks were not in the top-performing 10% in any strategy, being that Mulato II was placed on 24, 20, 14, 21, 20 and 20th, Marandu on 43, 51, 67, 70, 59 and 44th, and BRS Paiaguás on 88, 73, 92, 97, 93 and 88th in the ranking of strategies 1, 2, 3, 4, 5 and 6, respectively. These results also demonstrate the superiority of some new promising hybrids compared to

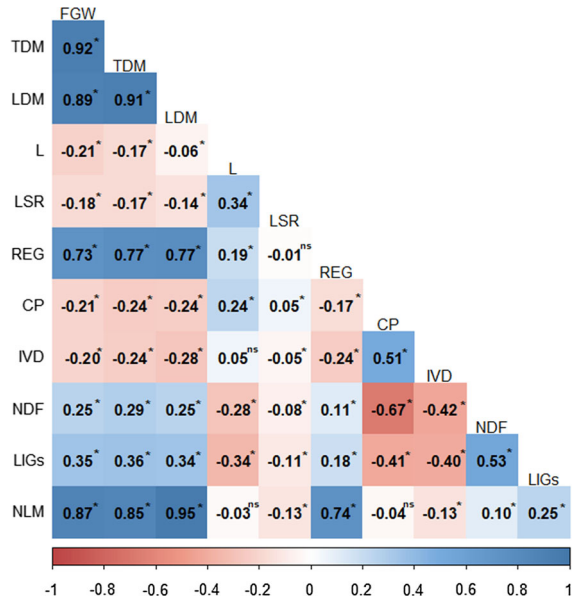

Fig. 1 Pearson correlations among BLUPs of the hybrids for field green weight (FGW), total dry matter (TDM), leaf dry matter (LDM), leaf percentage (L), leaf/stem ratio (LSR), regrowth (REG), crude protein (CP), in vitro digestibility of organic matter (IVD), neutral detergent fiber (NDF), lignin in sulfuric acid (LIG) and yield of high nutritional value leaf mass (NLM) in the combined analysis. *significant at 5% and ^{ns}not significant at 5% probability by the Bootstrap BCa method based on 9999 simulations. (Color figure online)

the commercial cultivars. By biplot analysis, the ten best genotypes based on the NLM-trait combination were 233, 778, 70, 242, 1130, 658, 1243, 421, 801 and 1221 (Fig. 3), being these the top-performing 10% in the direct selection as well. However, by the "which-won-where" biplot, it is possible to describe the profile of the genotypes in a more detailed manner, in which the genotype 233 proved to be superior in the combinations of NLM with nearly all the traits, except for LSR (Fig. 4), whereas the genotypes 778 and 1243 had a different profile, and were superior for the combination of NLM with LSR.

Discussion

Genetic parameters

The existence of genetic variation for the traits allows gain to be obtained from selection (Pandolfi Filho et al. 2016). However, the proportion of this genetic variation related to phenotypic variation or heritability varies according to the type of trait evaluated, the estimation method, diversity in the population (genetic variation), and various other factors (Dias et al. 2018; Schmidt et al. 2019). Matias et al. (2018) worked with a population of 1000 interspecific hybrids of *Urochloa* spp., and the heritability estimates obtained were very similar to those found in this study, except for LSR, which exhibited zero genetic variance. The heritabilities reported by Figueiredo et al. (2012) in *U. humidicola* hybrids were also similar for most of the traits, except for %L (68.32%) and IVD (63.93%). Therefore, the heritabilities in our study indicate the possibility to get high genetic gains with a selection of the best genotypes for most of traits.

Another highly relevant effect for forage breeding is the genotype x cutting interaction. The significant $\sigma^2_{G\times C}$ has implications for selection and has frequently been reported by other authors working with tropical forage species, such as *U. decumbens* and *U. humidicola* (Figueiredo et al. 2012; Mateus et al. 2015; Matias et al. 2016). Evidence of this interaction denotes that the genotypes under testing have a differential response to environmental fluctuations over the performed cuttings. The genotype x cutting interaction might have an impact on selection since genotypes may differ in terms of agronomic stability throughout the evaluation cuts, and it may also affect association among traits.

Estimates of genetic correlations that are of moderate magnitude and positive between CP × IVD and NDF × LIG have been reported in the literature, as well as correlations that are of moderate magnitude and negative between CP × NDF, CP × LIG, IVD × NDF, and IVD × LIG (Matias et al., 2016; Torres et al. 2016). The correlations estimated in the present study were similar, except for correlations between CP × IVD and CP × LIG, which were surprisingly statistically insignificant. In general, for the agronomic traits, the magnitudes of genetic correlations found in this study were similar to those observed in studies with *U. decumbens* and interspecific genotypes (Mateus et al. 2015; Matias et al. 2018). Furthermore, the estimates of the genetic correlations of the agronomic traits with the nutritional value traits CP, IVD, and NDF were low or zero, thus it is possible to select individuals that combine good agronomic performance and good nutritional quality. Similar estimates were found by Matias et al. (2018), who

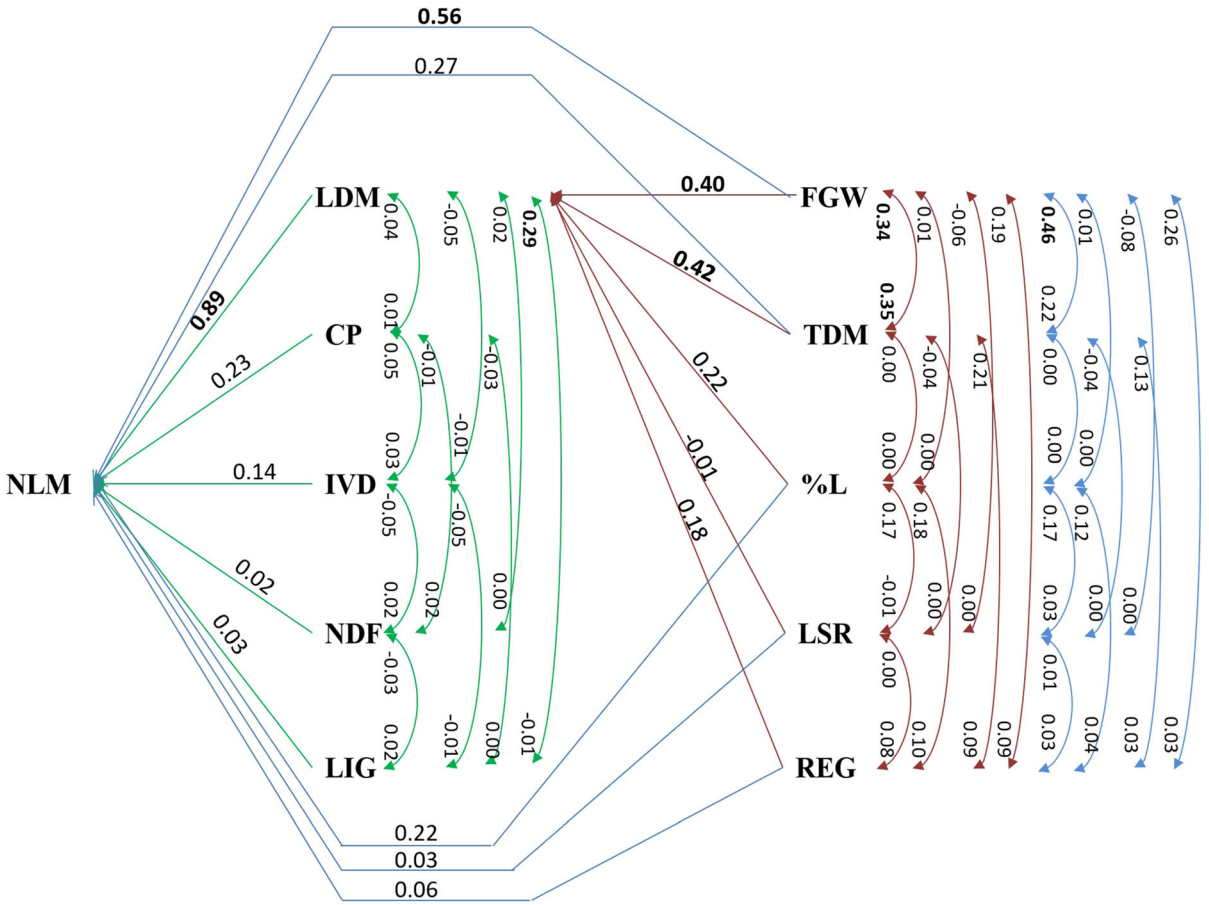

Fig. 2 Causal diagram of the direct and indirect effects of the secondary components of field green weight (FGW), total dry matter (TDM), leaf percentage (%L), leaf/stem ratio (LSR), and regrowth (REG), and of the primary component of leaf dry matter (LDM), crude protein (CP), in vitro digestibility of organic matter (IVD), neutral detergent fiber (NDF), and lignin in sulfuric acid (LIG) on yield of high nutritional value leaf mass (NLM). Single directional arrows indicate direct effects of the explanatory variables, whereas bidirectional arrows indicate indirect effects. Green lines indicate the direct and indirect effects of the primary variables on NLM, blue lines the direct and indirect effects of the secondary variables on NLM, and red lines the direct and indirect effects of the secondary variables on LDM. Numbers in bold print indicate values superior to the effect of the residual variable. (Color figure online)

studied interspecific hybrids of *Urochloa*, contrasting to those found in studies with *U. humidicola*, *U. decumbens*, and *U. ruziziensis*, where the increase in production of forage mass increased NDF and decreased CP and IVD (Figueiredo et al. 2012; Matias et al. 2016; Simeão et al. 2016). Matias et al. (2018) suggested that the presence of alleles of *U. ruziziensis* of high nutritional value combined with the alleles of good agronomic production of *U. brizantha* and *U. decumbens*, allowed for an equilibrium or independence of these groups of traits in the interspecific genotypes of *Urochloa*.

Genetic correlations are important in the choice of traits to be evaluated and in use of indirect selection (Matias et al. 2016). In this respect, FGW, TDM, and LDM stood out in this experiment, with high and positive correlation estimates with each other and with NLM, indicating that some of these traits can be used to perform indirect selection for NLM.

Path analysis

It is important to identify the traits of high correlation with the trait targeted in breeding or the basic variable, but, above all, those that have a greater direct effect in

Table 2 Gain from selection (%) for each trait using different selection strategies[b] of *Urochloa* hybrids in several cuttings[a], with a selection intensity of 10%

Str[b]	Traits used	Gain from selection (%)										
		FGW	REG	TDM	LDM	%L	LSR	CP	IVD	NDF	LIG	NLM
1	NLM	27	15	20	31	3	5	1	0	0	2	34
2	FGW	31	12	18	26	1	− 2	1	0	0	2	29
3	FGW and REG	27	21	18	29	3	6	− 1	0	0	3	27
4	ALL	22	19	14	28	5	14	1	1	− 1	1	31
5	ALL	29	16	19	30	3	6	2	0	− 1	1	33
6	ALL	27	15	20	31	3	5	1	0	0	2	34

FGW = field green weight (Kg ha^{-1}), *TDM* = total dry matter yield (Kg ha^{-1}), *LDM* = leaf dry matter yield (Kg ha^{-1}), *%L* = leaf percentage (%), *LSR* = leaf/stem ratio, *REG* = final regrowth capacity (score), *CP* = crude protein contents in dry matter (%), *IVD* = in vitro digestibility of organic matter (%), *NDF* = percentage of neutral detergent fiber (%), *LIG* = percentage of lignin in dry matter (%), *NLM* = yield of high nutritional value leaf mass (Kg ha^{-1})

[b]Strategies = 1: direct selection for NLM; 2: indirect selection for FGW; 3: index with weight of 60% for FGW and 40% for REG; 4: index including all the traits, except NLM, and weight of 60% for the agronomic traits and 40% for those of nutritional value; 5: index including all the traits and weight based on the direct effect of the traits on the NLM; 6: selection based on GYT analysis. [a]Seven cuttings: FGW, REG; six cuttings: TDM; three cuttings: LDM, %L, LSR, CP, IVD, NDF, LIG, and NLM

Table 3 Spearman correlation coefficient of the *Urochloa* hybrids between the different selection strategies[b]

Str[b]	1	2	3	4	5	6
1	1.00	0.82	0.83	0.84	0.95	0.99
2		1.00	0.80	0.62	0.88	0.80
3			1.00	0.85	0.91	0.85
4				1.00	0.87	0.88
5					1.00	0.96
6						1.00

[b]Strategies = 1: direct selection for NLM; 2: indirect selection for FGW; 3: index with weight of 60% for FGW and 40% for REG; 4: index including all the traits, except NLM, and weight of 60% for the agronomic traits and 40% for those of nutritional value; 5: index including all the traits and weight based on the direct effect of the traits on NLM; 6: selection based on GYT analysis

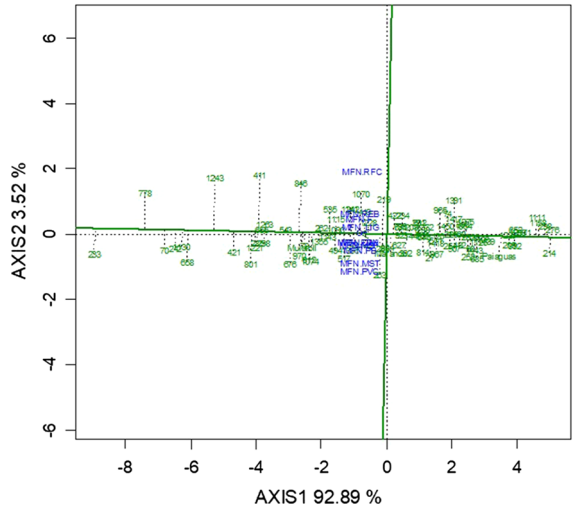

Fig. 3 View of mean coordination of the tester of the genotypes by yield*trait biplot constructed using breeding values of each genotype in combined analysis of the cuttings. The biplot was constructed using scaling by standard deviation, centered by 2, and SVP = JK—(row metric preserving). The codes of the traits are FGW: field green weight; TDM: total dry matter; L: leaf percentage; LSR: leaf/stem ratio; REG: regrowth; LDM: leaf dry matter; CP: crude protein; IVD: in vitro digestibility of organic matter; NDF: neutral detergent fiber; LIG: lignin in sulfuric acid; NLM: yield of high nutritional value leaf mass. (Color figure online)

a direction favorable to selection, so that the response correlated by means of indirect selection becomes efficient (Cruz et al. 2012). Thus, the results indicate that indirect selection for NLM by means of LDM and FGW is feasible and interesting.

Borges et al. (2011) performed path analysis in *U. ruziziensis* considering dry matter weight (equivalent to TDM) as the main trait, and nine traits in the primary chain, including stem and leaf dry weight, which were derived from fresh matter weight (FMW equivalent to FGW). The authors obtained high magnitudes of the indirect effects through stem and leaf dry weights for FMW, and when these two traits

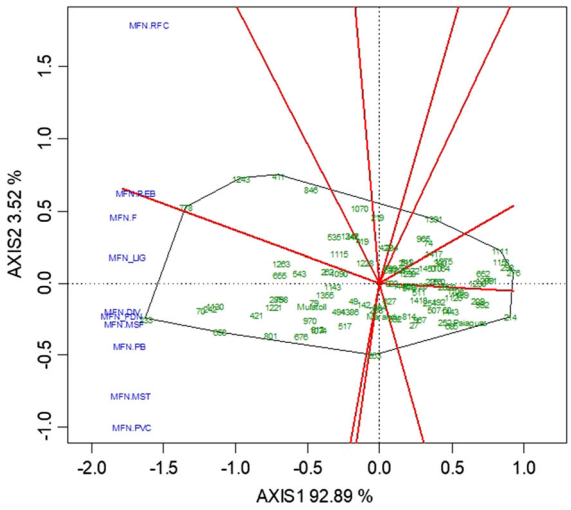

Fig. 4 Genotype by yield*trait biplot "which-won-where" constructed using breeding values of each genotype in combined analysis of the cuttings. The biplot was constructed using scaling by standard deviation, centered by 2, and SVP = SQ (Symmetrical). The codes of the traits are FGW: field green weight; TDM: total dry matter; L: leaf percentage; LSR: leaf/stem ratio; REG: regrowth; LDM: leaf dry matter; CP: crude protein; IVD: in vitro digestibility of organic matter; NDF: neutral detergent fiber; LIG: lignin in sulfuric acid; NLM: yield of high nutritional value leaf mass. (Color figure online)

were removed from path analysis, most of their indirect effects migrated to the estimate of the direct effect of FMW on the main trait. In our study, the high indirect effects via TDM for the FGW variable and via FGW for TDM can be explained by the fact that TDM is derived from FGW. Thus, both FGW and TDM can be used for indirect selection for LDM.

Selection strategies

Selection for FGW showed gain for NLM, confirming the possibility of carrying out indirect selection and corroborating the result of the direct effect indicated by path analysis. Selection based on FGW and REG, for its part, showed gain for NLM and high correlation (0.91) with the strategy that provided for an effective gain for NLM by indirect selection (strategy 5), suggesting that selection based on FGW and REG is also efficient for obtaining gains in NLM.

In spite of the favorable gains to NLM and this trait being a product of LDM, CP, and IVD, selection of 10% of the superior individuals based only on FGW resulted in a null gain for IVD, and selection based on FGW plus REG resulted in an unfavorable and null gains for CP and IVD, respectively. These null gains for IVD (strategies 2 and 3, Table 2) and unfavorable gain for CP (strategy 3, Table 2) suggest that LDM, as expected, had a greater influence on calculation of the NLM index. It should be noted that selection based on NLM is not expected to contribute to the increase in undesirable protein fractions, such as those connected with fiber, whose availability may be low in digestion by ruminants (Sniffen et al. 1992).

In a population of *U. humidicola*, selection aiming at greater forage production resulted in genotypes with lower crude protein contents (Figueiredo et al. 2012). However, in this study, it was shown that genotypes with greater gains in high nutritional value leaf mass can be selected through selection for FGW or FGW plus REG. These results are very important, since FGW and REG are traits that are more easily measured, and this will result in savings in time and resources for the forage breeding program, especially in the initial steps of the breeding program, in which thousands of hybrids are evaluated.

A common difficulty in plant breeding programs concerning simultaneous selection for several traits is the establishment of economic weights (Mateus et al. 2015). These weights can be predefined in a subjective way by the breeder, as was done for selection strategies 3 and 4. In the case of strategy 4, weight totaling 60% was chosen for agronomic traits and 40% for nutritional value traits, based on experience accumulated from previous evaluations of experiments on *Urochloa* spp., as well as observing the relative variation of the traits and their effect on the target trait of the breeding program.

One way of removing subjectivity in pre-establishment of economic weights is determination in an analytic manner. A reasonable proposal consists in using the relative direct effects to the trait or basic variable NLM, determined from path analysis, such as economic weights. The strategy or selection index 5 was based on this proposal and achieved an effective magnitude of gain for the NLM variable, being superior than the strategies of indexes with predefined weights (strategies 3 and 4), as well as high classification correlation of the genotypes compared to direct selection for NLM and with strategy 6.

GYT analysis allows selection for multiple traits to be performed without the need for assigning subjective weights to the traits (Yan and Frégeau-Reid 2018). This strategy achieved the highest magnitude of gain

for the NLM variable, equal to direct selection to NLM, and the greater *Spearman* correlation estimate of the genotypes with direct selection for NLM. It should be noted that the GYT strategy allows ranking and description of the profiles of the genotypes based on the combination of the main trait or basic variable, in this case NLM, with the other traits by means of biplots. Above all, this makes it easier to interpret and understand the results (Yan and Frégeau-Reid 2018).

Nevertheless, although the index with weights based on the direct effect on NLM and of GYT analysis show the greatest gains for NLM, there is the notable difficulty of application in practice due to the requirement of measuring varied traits, which may considerably raise the costs and time of phenotyping. Thus, the viability of these strategies is dependent on refining them and reducing costs on phenotyping, as well as in the use of equipment such as NIRS. These strategies might be more feasible at more advanced steps of the breeding program, in which there is fewer genotypes to be evaluated.

Superior hybrids than the checks were identified for NLM in this study. These hybrids were in the stage 1 of the breeding program where is also evaluated the reproductive mode (Jank et al. 2014), being that 233, 658, 1243, 421, 801 and 1221 were identified as sexual and 778, 70, 242 and 1130 as apomictic. The apomictic hybrids are new cultivar candidates whereas the sexual one new parent candidates for the future cycles of the breeding program. However, the hybrids must also be superior regarding spittlebug resistance and seed production. These traits are also evaluated in the stage 1 of the breeding program, and them the promising hybrids will proceed for next stages of evaluation, such as regional trials and value of cultivation and use (VCU) trials under artificial cutting and grazing until releasing a new cultivar.

Conclusion

The traits LDM and FGW exhibited the greatest direct effects on yield of high nutritional value leaf mass. The strategy of genotype by yield*trait analysis and the selection index with weights corresponding to the relative direct effects to each trait on the NLM resulted in superior correlated responses for NLM. The strategies of indirect selection via FGW and of the index including only FGW and regrowth proved to be viable for selection of genotypes of *Urochloa* in the initial steps of the breeding cycles because they associated practicality of use and high gain for NLM.

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